

=> d bib ab 26 34 37 39 40 42 43 49 53 56 60 61 64

L38 ANSWER 26 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1995-044064 [07] WPIDS

DNC C1995-019942

TI New alanine **racemase** from *Tolypocladium niveum* - used in enzymatic synthesis of D-amino acids.

DC B05 D16 E19

IN HOFFMANN, K; KLEINKAUF, H; PALMA, N; SANTER, G; SCHNEIDER-SCHERZER, E; SCHROEGENDORFER, K; ZOCHER, R

PA (SANDOZ) SANDOZ PATENT GMBH

CYC 1

PI DE 4314611 A1 19950112 (199507)* 11p

ADT DE 4314611 A1 DE 1993-4314611 19930504

PRAI DE 1993-4314611 19930504

AB DE 4314611 A UPAB: 19960520
Purified alanine **racemase** (I) from *Tolypocladium niveum* is new.
USE - (I) is used for enzymatic synthesis of D-amino

acids.

ADVANTAGE - (I) has sufficient thermal stability for use as biocatalyst and is able to epimerise most known amino acids (with varying degrees of efficiency).

Dwg. 0/0

L38 ANSWER 34 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1991-159796 [22] WPIDS

DNC C1991-069067

TI New aspartic acid **racemase** - catalyses racemisation of D- and L-aspartic acids and is obtained from *streptococcus thermophilus* - based on methionine, glutamic acid, asparagine, phenylalanine, serine, isoleucine, leucine, glycine, threonine, etc..

DC B04 D16

PA (ASAG) ASAHI GLASS CO LTD

CYC 1

PI JP 03094678 A 19910419 (199122)* Sp

JP 2950865 B2 19990920 (199944) Sp

ADT JP 03094678 A JP 1989-231395 19890908; JP 2950865 B2 JP 1989-231395 19890908

FDT JP 2950865 B2 Previous Publ. JP 03094678

PRAI JP 1989-231395 19890908

AB JP 03094678 A UPAB: 19930928
Novel aspartic acid **racemase** (a) catalyses racemic reaction from L-aspartic acid into D- and L-aspartic acid and racemic reaction from D-aspartic acid into L- and D-aspartic acid, (b) acts selectively on aspartic acid racemic reaction and does not act on other amino acid, (c) has optimum pH of 8 to 37 deg.C, (d) shows no redn. in activity after 1 hr. treatment at 45 deg.C at pH 6.5-8, (e) shows no

redn. in activity by treating at 50 deg.C for 60 mins. at pH 7, (f) has total molecular wt. of about 60000 (gel filtration) and subunit molecular wt.

of

28000, and (g) has amino acid sequence of Met-Glu-Asn-Phe -Phe-Ser-Ile-Leu -Gly-X-Met-Gly -Thr-Met-Ala-Thr -Glu-Ser-Phe at the terminal.

The **racemase** is obtd. by cultivation of *Streptococcus* thermophilus IAM 10064.

0/0

L38 ANSWER 37 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1990-255666 [34] WPIDS

DNC C1990-110656
TI Racemisation of aminoacid amide cpds. - by contact with enzyme with
aminoacid amide **racemase** activity from microorganism.

DC B05 D16 E19
IN HERMES, H; PEETERS, W; PETERS, P H
PA (NOVO) NOVO NORDISK AIS; (STAM) STAMICARBON BV
CYC 9

PI EP 383403 A 19900822 (199034)*
R: BE CH DE ES FR GB IT LI NL
ADT EP 383403 A EP 1990-200335 19900214
PRAI EP 1989-200380 19890216; EP 1990-200335 19900214
AB EP 383403 A UPAB: 19930928

Process for complete or partial racemisation of aminoacid amides of
formula R-CH(NH2)-CONH2 (I) is claimed which comprises exposing the cpds.
to an enzyme with aminoacid amide **racemase** activity, which
enzyme is in species of the genus Klebsiella and related genera e.g.
Enterobacter, Escherichia, Shigella, Citrobacter and Salmonells from the
family of the Enterobacteraceae. (R = indolyl, benzyloxy, lower alkyl
opt. substd. by OH, SH, amino, halogen, phenyl, phenoxy, benzyl or lower
alkylthio, or R = phenyl opt. substd. by one or more of OH, amino,
halogen,

carboxy or lower alkoxy). Pref., the enzyme is derived from Klebsiella
oxytoca strain NCIB 40113, Pseudomonas putida strain ATCC 12633 or NCIB
40042 or Rhodococcus sp. strain NCIB 40041.

USE/ADVANTAGE - Aminoacid amides can be racemised with low losses of
material through by-prod. formation. The prods. can be subjected to
enantioselective enzymatic hydrolysis to prepare optically active
amino acids which can be used as e.g. food and feed
additives. @
0/0

L38 ANSWER 39 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1990-189443 [25] WPIDS

DNC C1990-082123
TI Racemisation of aminoacid - using **racemase** contg. microbial
cells which are pre-contacted with cell wall digesting enzyme.

DC B05 D16 E19
PA (KEIS) KEISHITSU RYUBUN SHINYOTO

CYC 1
PI JP 02124097 A 19900511 (199025)*
ADT JP 02124097 A JP 1988-276050 19881102
PRAI JP 1988-276050 19881102
AB JP 02124097 A UPAB: 19930928

In racemisation of **amino acid** by using **amino acid racemase** contg. microbial cells or their
immobilised substance, the cells or their immobilised substance are
preliminary contacted with cell wall digesting enzyme.
Pref. cell wall digesting enzyme is glycosidase, pref. lysozyme or
N-acetyl muramidase. The **amino acid racemase**

contg. microbe is sensitive to glycosidase.
USE/ADVANTAGE - Cell membrane permeability of cells can be increased
under conditions mild enough to maintain activity without damaging
racemase activity. Thus **amino acid** can be
racemised in high yield.

0/0

L38 ANSWER 40 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1990-189437 [25] WPIDS

DNC C1990-082117
TI Culture of pseudomonas S.P. bacteria - has low substrate specificity and
contains **amino acid racemase** one or more of
L- or DL-glutamic acid.

DC B04 D16
PA (KEIS) KEISHITSU YUBUN SHINYOTO
CYC 1
PI JP 02124087 A 19900511 (199025)*
ADT JP 02124087 A JP 1988-276052 19881102
PRAI JP 1988-276052 19881102
AB JP 02124087 A UPAB: 19930928
In culturing Pseudomonas s.p. bacteria which has low substrate specificity and contains **amino acid racemase** one or more kinds of L- or DL-glutamic acid and its salts, L- or DL-aspartic acid or its salts, L- or DL-alanine, L- or DL-serine, L- or DL-lysine and its hydrochloride, L- or DL-proline, L- or DL-leucine and L- or DL-arginine, are used as carbon source.

USE/ADVANTAGE - High content **racemase** retained cells can be obt'd. without inhibiting **racemase** formation in cells, **amino acid** can be racemised in high efficiency with using the cells.

In an example, each 100 ml of medium (polypeptone, meat extract, NaCl, distilled water 1000 ml) was sterilised. To this, 1 loop of *Pseudomonas putida* IFO122996 was inoculated and shaking cultured at 30 deg.C for 15 hours. Then each 1 ml of the culture was inoculated to each 100 ml of medium (K2HPO4, KH2PO4 (NH4)2SO4, MgSO4.7H2O, tryptone (Difco), yeast extract (Difco), principal C sources, e.g. Na L-glutamate, Na L-aspartate, L-alanine, L-serine, L-lysine.HCl, L-proline, L-leucine or L-arginine, distilled H2O 1000 ml, pH 7.0), and spinner cultured at 30 deg.C for 20 hours. Cells were collected from each culture (40 ml) by centrifugation, washed with 0.1M phosphate buffer, then suspended in the same buffer and ultrasonificated, centrifuged to obtain crude enzyme soln..

0/0

L38 ANSWER 42 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1990-152258 [20] WPIDS
DNC C1990-066460
TI Culturing method for **amino acid racemase** -producing microbe - by keeping nitrogen source concn. in culture medium at specific concn..

DC B04 B05 D16 E19
PA (KEIS-N) KEISHITSURYUBUN SHI
CYC 1
PI JP 02097377 A 19900409 (199020)*
ADT JP 02097377 A JP 1988-249771 19881005
PRAI JP 1988-249771 19881005
AB JP 02097377 A UPAB: 19930928
Culturing the bacterial strain which belongs to *Pseudomonas* and produces **amino acid racemase** (abbr. AAR) and is characterised by keeping the N source concn. in culture medium in the range

of 0.005-0.02 w/w% as N concn.
Pref. as N source ammonium salts, nitrate, organic cpds. such as glutamic acid, glutamine, aspartic acid, asparagine, etc. can be used and for keeping N source concn. they are added continuously or intermittently.

USE/ADVANTAGE - For synthesising **amino acids** economically, racemisation process is required and the enzymic racemisation by AAR is desirable. It has been known that *Pseudomonas putida* IFO12996 can produce AAR and its AAR productivity is very low. Thus the culturing method for increasing the prodn. of AAR has been expected and a culturing method has been obt'd. using glycerol, ethanol, lactic acid, citric acid, etc. as carbon source (pat. publ. No. 205781/87). Also keeping N source concn. in culture medium in above range,

the bacterial body showing high AAR acrivity can be obt'd. @@

TI Pseudomonas putida IFO 12996 aminoacid **racemase** prodn. - using glycerol, ethanol and/or tartaric acid as carbon source, giving increased enzyme prodn. relative to glucose.

DC B05 D16 E16
IN ENDO, F; SHIMAZU, M; YUKAWA, H
PA (REUT-N) RES ASSOC UTIL LIGH

CYC 1
PI US 4906572 A 19900306 (199016)*
ADT US 4906572 A US 1987-21356 19870303
PRAI JP 1986-44122 19860303
AB US 4906572 A UPAB: 19930928

Prodn. of an **amino acid racemase** comprises culturing pseudomonas putida IFO 12996 in a culture medium contg. glycerol, ETOH and/or tartaric acid as carbon source, the total concn. of carbon source added to the medium during cultivation being at least 0.5% w/v, and recovering cells contg. the **racemase**.

USE/ADVANTAGE - Used for racemisation of residual D-**amino**

acids in synthesis of L-**amino acids** via racemic forms, the Ps. putida **racemase** being active on eg. lysine, arginine, methionine, alanine or serine. Prodn. of the **racemase** is increased using the specified carbon sources (c.f. glucose which inhibits enzyme prodn.) making prodn. more favourable industrially.

0/0

TI New acyl aminoacid **racemase** for optically active aminoacid prodn. - from racemic N-acylamino-carboxylic acid in presence of specific amino acylase.

DC B04 B05 D16 E19
IN HATANO, K; TAKAHASHI, T
PA (TAKE) TAKEDA CHEM IND LTD

CYC 19
PI EP 304021 A 19890222 (198908)* EN 20p
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
HU 47317 T 19890228 (198914)
DK 8804624 A 19890222 (198920)
JP 01137973 A 19890530 (198927)
CN 1035320 A 19890906 (199028)
US 4981799 A 19910101 (199104) 13p
EP 304021 B1 19930428 (199317) EN 22p
R: AT BE CH DE ES FR GB GR IT LI LU NL SE

DE 3880585 G 19930603 (199323)
JP 2712331 B2 19980210 (199811) 12p

KR 9700185 B1 19970106 (199932)
ADT EP 304021 A EP 1988-113315 19880817; JP 01137973 A JP 1988-180778
19880720; US 4981799 A US 1988-227882 19880803; EP 304021 B1 EP
1988-113315 19880817; DE 3880585 G DE 1988-3880585 19880817, EP
1988-113315 19880817; JP 2712331 B2 JP 1988-180778 19880720; KR 9700185

B1 KR 1988-10620 19880820
FDT DE 3880585 G Based on EP 304021; JP 2712331 B2 Previous Publ. JP 01137973

PRAI JP 1987-208484 19870821; JP 1988-180778 19880720

AB EP 304021 A UPAB: 19930923
The new enzyme acylamino acid **racemase** (AAR) converts D-N-acyl-alpha-aminocarboxylic acid (I) to the corresponding L-isomer, and vice versa, but does not convert D-alpha-amino acid to its L-isomer, nor vice versa. (I) has formula X-NH-CHR-COOH; X = opt. subst. carboxylic acyl,

best

1-3C alkanoyl or benzoyl opt. substd. by halo, 1-3C alkyl, 1-3C alkoxy and/or NO₂; R = [REDACTED] substd. 1-20C alkyl, esp. 1-[REDACTED] unsubstd.; 1-3C substd. by OH, 1-3C alkylthio, SH, phenyl, hydroxyphenyl or indolyl; or 1-4C substd. by NH₂, COOH, guanidino or imidazolyl.

USE/ADVANTAGE - AAR is used to produce D-orL-amino acids by treatment of DL- (I), in presence of D-orL- aminoacylase (AA). This process provides 100% conversion of starting material to a specific amino acid without sepn. or racemisation steps.

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L38 ANSWER 53 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1988-333485 [47] WPIDS

DNC C1988-147240

TI New amino acid racemate - is derived from Pseudomonas, and used for racemising aromatic amino acids.

DC B05 D16
PA (SAGAMI) SAGAMI CHEM RES CENTRE

CYC 1
PI JP 63245673 A 19881012 (198847)* 11p
JP 07089928 B2 19951004 (199544) 8p

ADT JP 63245673 A JP 1987-76290 19870331; JP 07089928 B2 JP 1987-76290 19870331

FDT JP 07089928 B2 Based on JP 63245673

PRAI JP 1987-76290 19870331

AB JP 63245673 A UPAB: 19930923

Amino acid racemase has the following characteristics: (1) Catalyses reaction of generation of racemate from L- or D-amino acids. (2) Shows wt. of 60,000-80,000 by HPLC gel filtration and mol. wt. of subunit of 30,000-47,000 by SDS-PAGE. (3) Acts on phenylalanine, tryptophan and tyrosine. Pref. any strain of Pseudomonas with **racemase** activity can be used, but pref. strain is Pseudomonas putida SCRC-744 (FERM P-9039) derived from a soil of Kanagawa prefecture. Relative **racemase** activity to D-amino acids is D-glutamine (100%), D-methionine (42%), D-alanine (36%), D-serine (31), D-lysine (22), D-arginine (21%), D-leucine (15%), D-asparagine (6%), D-cysteine (6%), D-histidine (5%), D-phenylalanine (1%), D-tryptophan (0.8%) and D-tyrosine (0.4%). The **racemase** can act on aromatic D-amino acids. optimum pH and temp. are pH 8 and 50-60 deg. C, respectively.

USE/ADVANTAGE - The **racemase**, different from known racemases, racemises aromatic amino acids and does not need ATMP as a coenzyme. The **racemase** is new and is derived from Pseudomonas.

0/5

L38 ANSWER 56 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

AN 1987-300108 [43] WPIDS

DNC C1987-127687

TI Aminoacid **racemase** from Pseudomonas - by growing on medium contg. non-sugar carbon source, esp. glycerol.

DC B05 D16 E19
IN ENDO, F; SHIMAZU, M; YUKAWA, H

PA (KEIS-N) KEISHITSU-RYUBUN SH; (REAS-N) RES ASSOC UTIL LIGH

CYC 2
PI DE 3706724 A 19871022 (198743)* 5p

JP 62205781 A 19870910 (198743)

ADT DE 3706724 A DE 1987-3706724 19870302

PRAI JP 1986-44122 19860303

AB DE 3706724 A UPAB: 19930922
Pseudomonas microorganisms which produce amino acid **racemase** (I) are grown in a culture medium contg. glycerol, EtOH, tartaric-, fumaric- or succinic-acids as C source, then cells, which

contain large amts of (I), recovered. Pref the C source is glycerol and the total concn C source added during cultivation is at least 0.5 wt./vol.%. The microorganism is esp P putida IFO 12996. Pref the microorganism is grown at 10-45, (25-40, deg C and pH 3-10, (5-9), under aerated conditions for 4 hr - 3 days. The C source can be added to the starting medium, or added in portions during the incubation. The harvested cells can be used directly (wet or dry) or they can be lysed and opt. a cell-free, enzyme-contg. extract recovered.

USE/ADVANTAGE - This method provides higher yield of (I) than is possible using glucose as C source. (I) is esp used to racemise residual D-amino acids left after recovery of the L-isomer.
0/0

L38 ANSWER 60 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1987-153956 [22] WPIDS

DNC C1987-064352
TI L-Isoleucine prepn. from D or DL amino butyric acid - in presence of enzyme racemising the acid or a microorganism contg. enzyme.

DC B05 D16 E16
PA (MITSUB) MITSUBISHI PETROCHEMICAL CO LTD

CYC 1
PI JP 62091191 A 19870425 (198722)* 4p

ADT JP 62091191 A JP 1985-232081 19851017

PRAI JP 1985-232081 19851017

AB JP 62091191 A UPAB: 19930922

D-alpha-aminobutyric acid or DL-alpha-aminobutyric acid is added to a culture medium and a bacterium is cultured in the medium in presence of an enzyme which racemises D-alpha-aminobutyric acid or a microorganism contg.

the enzyme or treatment of the microorganism. *before back p. 37, 38*

Pref. L-isoleucine-producing bacteria are Brevibacterium flaccum
MJ-233 (ferm-P 3068) and Brevibacterium flaccum MJ-233-AB-41 (FERM-P

3812).

They are cultured in a medium (pH 7-8) at 25-35 deg.C for 5 days under aerobic conditions.

The enzyme which can racemise D-alpha-aminobutyric acid is produced by Pseudomonas putida (IFO 12996). The enzyme may be added from the first or in course of the culture. After the culture, bacterial cells are removed

and L-isoleucine is purified from the culture medium as usual.

USE/ADVANTAGE - L-isoleucine, one of essential amino acids, has been produced by fermentation using DL-alpha-aminobutyric acid as precursor, because DL-alpha-aminobutyric acid is easily available. The prod. rate of L-isoleucine from D-alpha-aminobutyric acid. In this method the prodn. rate from D-alpha-aminobutyric acid is increased by adding a racemase in the medium and yield of L-isoleucine is high.

0/0

L38 ANSWER 61 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1986-276390 [42] WPIDS

DNC C1986-119658
TI Gene coding alpha-amino-epsilon-caprolactam racemase - used in conversion of D- or DL- cpd. to L-lysine by recombinant DNA.

DC B04 D16
PA (TORA) TORAY IND INC

CYC 1
PI JP 61202693 A 19860908 (198642)* 9p

ADT JP 61202693 A JP 1985-42739 19850306

PRAI JP 1985-42739 19850306

AB JP 61202693 A UPAB: 19930922

Gene coding alpha-amino-epsilon-caprolactam racemase whose M.W. is 4.5×10 power 4 dalton and amino acid sequence of

N-terminal side is: Thr-Lys-Ala-Leu-Tyr-Asp-Arg-Asp-Gly
-Ala-Ala-Ile-Gly-L-¹-Leu-Gly-Lys-Leu -Arg-Phe-²-Leu-Ala-Ile-Ser-Gly
-Gly-Arg-Gly-Ala-L-³-Leu-Ile-Glu-Glu -Asn-Gly-Arg-L-⁴-Leu-Ile-Asp-Leu-Ser
-Gly-Ala 3.5Kb DNA fragment contg. the gene. Recombinant DNA obtd. by
integrating the DNA fragment is also claimed.

DNA donor is Achromobacterium obae having alpha-amino-epsilon-
caprolactam racemase producibility. The DNA fragment is obtd. by
general method. The recombinant DNA is obtd. by binding the DNA fragment
with plasmid vector, transforming host with the plasmid, cultivating the
transformed cell in medium contg. L-alpha-amino-epsilon-caprolactam
hydrolase and D-alpha-amino-epsilon-caprolactam, selecting transformed
cell having the racemase activity and extracting plasmid. The
host is lysine-requiring mutant of E. coli. Amt. of the hydrolase is
10-100 unit/ml.

USE/ADVANTAGE - E. coli transformed by the recombinant DNA and
having alpha-amino-epsilon-caprolactam racemase producibility can
convert D- or DL-alpha-amino-epsilon-caprolactam to L-lysine by combined
use with L-alpha-amino-epsilon-caprolactam hydrolase.
0/0

L38 ANSWER 64 OF 64 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1980-54522C [31] WPIDS
TI Quantitative or qualitative determin. of aminoacid - involves converting
one optically-active isomer to another using aminoacid-**racemase**
and oxidn..
DC B05 D16
PA (MATU) MATSUSHITA ELEC IND CO LTD
CYC 1
PI JP 55081595 A 19800619 (198031)*
JP 56043358 B 19811012 (198145)
PRAI JP 1978-155633 19781214
AB JP 55081595 A UPAB: 19930902
The method for analysing, aminoacids quantitatively or qualitatively
involves converting L-(or d-) aminoacid to D-(or L-) aminoacid with
aminoacid-**racemase**; oxidising the converted D-(or L-) aminoacid
with D-(or L-) aminoacid-oxidase; and measuring the change during oxidn. By
this method specific aminoacid in the substance contg. various aminoacids
can be selectively analysed.

As amino acid-**racemase** alanine-
racemase, methionine-**racemase**, glutamate-
racemase, proline-**racemase**, lysine-**racemase**,
threonine-**racemase**, etc. can be used. Aminoacid-**racemase**
and D-(or L-) aminoacid-oxidase are pref. used in the form of fixed
enzyme. For the object the change during oxidn. is measured. The
decrease in oxygen concn. the increase in hydrogen peroxide concn., the
decrease in redox cpd. (oxidised form) concn. or the increase in redox
cpd. (reduced form) concn. can be used as the indicator, and they can be
easily measured electrically or colorimetrically.

=> d his

(FILE 'HOME' ENTERED AT 14:37:26 ON 23 MAR 2001)
FILE 'CA' ENTERED AT 14:37:54 ON 23 MAR 2001

L1 2245 S EPIMERASE OR RACEMASE
L2 450535 S AMINO ACID#
L3 611 S L1 AND L2
L4 89500 S ARTHROBACTER OR PSEUDOMONAS OR RHIZOBIUM OR STREPTOMYCES OR
N
L5 137 S L3 AND L4
L6 11842 S ISOMERASE
L7 13890 S L1 OR L6

L8 2256 S L7 AND L2
 L9 313 S L8 AND L4
 L10 1513 S EPIMERASE
 L11 450535 S AMINO ACID#
 L12 1513 S L10 AND L1
 L13 313 S L10 AND L11
 L14 3788 S D-AMINO ACID#
 L15 4 S L14 AND L10
 L16 5047 S L-AMINO ACID#
 L17 1 S L16 AND L10
 L18 8 S HYDROXYPROLINE EPIMERASE
 L19 3 S "E.C. 5.1.1.8"
 L20 10 S L18 OR L19
 L21 0 S AMINO ACID EPIMERASE

FILE 'BIOSIS' ENTERED AT 15:05:44 ON 23 MAR 2001

L22 0 S AMINO ACID EPIMERASE#
 L23 1236 S EPIMERASE#
 L24 251596 S AMINO ACID
 L25 125371 S AMINO ACIDS
 L26 321771 S L24 OR L25
 L27 173 S L23 AND L26
 L28 1818 S D-AMINO ACID
 L29 2111 S L-AMINO ACID
 L30 87521 S CONVERSION OR INVERSION OR EPIMERIZATION
 L31 12 S L28 AND L29 AND L30

FILE 'USPATFULL' ENTERED AT 15:22:32 ON 23 MAR 2001

L32 1255 S EPIMERIZATION OR EPIMERASE
 L33 72318 S AMINO ACID#
 L34 455 S L32 AND L33
 L35 78 S L32 (P) L33

FILE 'WPIDS' ENTERED AT 15:30:13 ON 23 MAR 2001

L36 183 S EPIMERASE OR RACEMASE
 L37 42642 S AMINO ACID#
 L38 64 S L36 AND L37

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O ANSWER 5 OF 10 CA COPYRIGHT 2001 ACS
AN 100:47729 CA
TI Hydroxyproline 2-epimerase of *Pseudomonas*. Subunit structure and active site studies
AU Ramaswamy, Sengoda G.
CS Sch. Med., Univ. Maryland, Baltimore, MD, 21201, USA
SO J. Biol. Chem. (1984), 259(1), 249-54
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Hydroxyproline 2-epimerase (I) of *P. putida* was purified to homogeneity by an improved procedure. Native I consisted of 2 probably identical subunits. Alkylation of the active site with labeled reagents resulted in the loss of 80-85% of the activity, but with the incorporation of only 1 alkyl group even though the active site contains a cysteine residue from each of the 2 subunits. This result suggested that I showed half-of-the-sites reactivity. The labeled enzyme was further subjected to exhaustive alkylation with unlabeled iodoacetate, permitting tryptic hydrolysis and isolation of an active site peptide in 30% yield. The specific radioactivity of the peptide was consistent with the 1st result, that only 1 mol of alkyl group was initially incorporated into the active site. The active site peptide (14 residues) was sequenced and found to possess homol. with the clostridial proline racemase.
CC 7-5 (Enzymes)
ST hydroxyproline epimerase structure *Pseudomonas*; subunit structure hydroxyproline epimerase *Pseudomonas*; active site hydroxyproline epimerase sequence
IT *Pseudomonas putida*
 (hydroxyproline epimerase of, active site and subunit structure of)
IT Mercapto group
 (in hydroxyproline epimerase active site)
IT Protein sequences
 (of hydroxyproline epimerase active site, of *Pseudomonas putida*)
IT 9024-23-1
 RL: BIOL (Biological study)
 (active site and subunit structure of, of *Pseudomonas putida*)
IT 52-90-4, biological studies
 RL: BIOL (Biological study)
 (in hydroxyproline epimerase active site)

L9 ANSWER 76 OF 313 CA COPYRIGHT 2001 ACS
AN 128:191643 CA

TI Racemization of serine with broad substrate specificity **amino acid racemase** immobilized in polyacrylamide gel

AU Seto, Takatoshi; Imanari, Makoto
CS Tsukuba Res. Center, Mitsubishi Chemical Co., Ltd., Ibaraki, 300-03,

Japan
SO Nippon Kagaku Kaishi (1997), (11), 784-789
CODEN: NKAKB8; ISSN: 0369-4577

PB Nippon Kagakkai
DT Journal
JA **Japanese**

AB Racemization of serine is an important reaction in the process of L-tryptophan synthesis from serine and indole. Broad substrate specificity **amino acid racemase** (EC 5.1.1.10) extd. from *Pseudomonas putida* was immobilized in polyacrylamide gel. Velocities of racemization of serine by both the native and immobilized enzymes were measured and analyzed. Two reactions catalyzed by both enzymes followed an equation of Michaelis-Menten type. For the native enzyme Michaelis const. (Km) of D-serine formation from L-serine was 4.1×10^{-2} M, being Km of the reverse reaction 1.8×10^{-2} M. Both Km and max. reaction velocity (Vm) for the forward reaction showed approx. two-fold larger than those for the reverse reaction. In the immobilized enzyme the real Kms were 1.1.-1.4 fold larger than those in the native enzyme, and the apparent Km increased with increasing a size of

gel. Vms in the immobilized enzyme were 60-70% of those in the native enzyme. Extent of variations of Km and Vm was almost the same in forward and reverse reactions. By the measurement of the activity of the immobilized enzyme in a flow-reactor within 23 days the activity maintained stably within exptl. errors.

=> d pn 1 76

L35 ANSWER 1 OF 78 USPATFULL
PI US 6204050 20010320

L35 ANSWER 76 OF 78 USPATFULL
PI US 4510246 19850409

DN BA90:20626
TI PURIFICATION AND CHARACTERIZATION OF THE ISOPENICILLIN N EPIMERASE
FROM NOCARDIA-LACTAMDURANS.
AU LAIZ L; LIRAS P; CASTRO J M; MARTIN J F
CS AREA MICROBIOL., DEP. ECOL. GENET. MICROBIOL., FAC. BIOL., UNIV. LEON,
LEON, SPAIN.
SO J GEN MICROBIOL, (1990) 136 (4), 663-672.
CODEN: JGMIAN. ISSN: 0022-1287.
FS BA; OLD
LA English
AB Isopenicillin N (IPN) **epimerase**, an enzyme involved in
cephalosporin and cephamycin biosynthesis that converts IPN into
penicillin N, was extracted from *Nocardia lactamdurans* and purified
88-fold. The enzyme was unstable but could be partially stabilized by
addition of pyridoxol phosphate. The purified enzyme did not require ATP
for activity in contrast to other **amino acid** racemases. The enzyme had an Mr of 59000 as determined by gel filtration;
IPN **epimerase** from *Streptomyces clavuligerus* had an Mr of 63000.
A protein band of Mr 59000 was found to be enriched in SDS-PAGE of active
fractions from *N. lactamdurans*. The optimal temperature of the
epimerase was 25 degree C and the optimal pH 7.0. The apparent Km
for IPN was 270 μ M. Fe^{2+} , Cu^{2+} , Hg^{2+} and Zn^{2+} strongly inhibited
enzyme activity. α -Aminoadipic acid, valine, glutamine, glycine, aspartic
acid and glutathione do not affect enzyme activity, whereas ammonium
sulphate was inhibitory. The **epimerase** activity was partially
inhibited by several thiol-specific reagents.

WARR
L27 ANSWER 152 OF 173 BIOSIS COPYRIGHT 2001 BIOSIS

AN 1990:88185 BIOSIS

DN BA89:47536

TI EXPRESSION OF RECOMBINANT DIAMINOPIMELATE EPIMERASE IN
ESCHERICHIA-COLI ISOLATION AND INHIBITION WITH AN IRREVERSIBLE
INHIBITOR.

AU HIGGINS W; TARDIF C; RICHAUD C; KRIVANEK M A; CARDIN A A
CS MERRELL DOW RES. INST., 16 RUE D'ANKARA, F-67009 STRASBOURG CEDEX, FR.

SO EUR J BIOCHEM, (1989) 186 (1-2), 137-144.

CODEN: EJBCAI. ISSN: 0014-2956.
FS BA; OLD
LA English
AB Recombinant diaminopimelate **epimerase** is overproduced to give 1%
of soluble protein when grown under the appropriate conditions in
Escherichia coli. This compares with 0.02% of the constitutive level of
wild-type enzyme. A new purification procedure now yields milligram
quantities of homogeneous enzyme of high specific activity (192 U/mg).
This has enabled sufficient amounts of enzyme both to compare with
wild-type enzyme and to enable active site modification studies to be
performed. Incubation of the enzyme with 2-(4-amino-4-carboxybutyl)-2-
aziridine-carboxylic acid (AZIDAP), results in time-dependent
irreversible
inhibition. Tryptic digestion of the inactivated enzyme and
peptide-mapping show that AZIDAP is specifically and covalently bound to
the enzyme at a unique peptide. Determination of the **amino acid**
sequence of this peptide and comparison with the sequence
deduced from the DNA sequence of the dapF gene shows that Cys73 is
labelled. Finally based on limited sequence similarities around this
cysteine and active-site cysteines of proline racemase and
1-hydroxyproline 2-**epimerase**, together with mechanism
considerations, we propose that all three non-pyridoxal-phosphate-
containing racemases/**epimerases** derive from a common
evolutionary origin.

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AN 1976:110098 BIOSIS

DN BA61:10098